Randomized, Double-Blind, Placebo-Controlled, Multicenter Phase II Study of Onartuzumab Plus Bevacizumab Versus Placebo Plus Bevacizumab in Patients With Recurrent Glioblastoma: Efficacy, Safety, and Hepatocyte Growth Factor and O(6)-Methylguanine-DNA Methyltransferase Biomarker Analyses

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ORIGINAL REPORT

Randomized, Double-Blind, Placebo-Controlled, Multicenter Phase II Study of Onartuzumab Plus Bevacizumab Versus Placebo Plus Bevacizumab in Patients With Recurrent Glioblastoma: Efficacy, Safety, and Hepatocyte Growth Factor and O^6-Methylguanine–DNA Methyltransferase Biomarker Analyses

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ABSTRACT

Purpose

Bevacizumab regimens are approved for the treatment of recurrent glioblastoma in many countries. Aberrant mesenchymal-epithelial transition factor (MET) expression has been reported in glioblastoma and may contribute to bevacizumab resistance. The phase II study GO27819 investigated the monovalent MET inhibitor onartuzumab plus bevacizumab (Ona + Bev) versus placebo plus bevacizumab (Pla + Bev) in recurrent glioblastoma.

Methods

At first recurrence after chemoradiation, bevacizumab-naïve patients with glioblastoma were randomly assigned 1:1 to receive Ona (15 mg/kg, once every 3 weeks) + Bev (15 mg/kg, once every 3 weeks) or Pla + Bev until disease progression. The primary end point was progression-free survival by response assessment in neuro-oncology criteria. Secondary end points were overall survival, objective response rate, duration of response, and safety. Exploratory biomarker analyses correlated efficacy with expression levels of MET ligand hepatocyte growth factor, O^6-methylguanine–DNA methyltransferase promoter methylation, and glioblastoma subtype.

Results

Among 129 patients enrolled (Ona + Bev, n = 64; Pla + Bev, n = 65), baseline characteristics were balanced. The median progression-free survival was 3.9 months for Ona + Bev versus 2.9 months for Pla + Bev (hazard ratio, 1.06; 95% CI, 0.72 to 1.56; $P = .7444$). The median overall survival was 8.8 months for Ona + Bev and 12.6 months for Pla + Bev (hazard ratio, 1.45; 95% CI, 0.88 to 2.37; $P = .1389$). Grade ≥ 3 adverse events were reported in 38.5% of patients who received Ona + Bev and 35.9% of patients who received Pla + Bev. Exploratory biomarker analyses suggested that patients with high expression of hepatocyte growth factor or unmethylated O^6-methylguanine–DNA methyltransferase may benefit from Ona + Bev.

Conclusion

There was no evidence of further clinical benefit with the addition of onartuzumab to bevacizumab compared with bevacizumab plus placebo in unselected patients with recurrent glioblastoma in this phase II study; however, further investigation into biomarker subgroups is warranted.

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ASSOCIATED CONTENT

Appendix
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INTRODUCTION

Standard treatment of newly diagnosed glioblastoma (the most common primary tumor in the CNS[1,2]) is surgical debulking followed by radiotherapy and temozolomide, providing a median overall survival (OS) of 14.6 months.[3,4] Recent data suggest that glioblastoma comprises clinically relevant subtypes (proneural, mesenchymal, and proliferative/neural) that exhibit different prognostic outcomes and treatment

responses.[5] Prognostic benefits have been reported for mesenchymal and neural subtypes, unlike proneural subtypes.[5]

Glioblastomas are highly vascularized tumors[6] and respond to antiangiogenic agents.[7-13] The Avastin in Glioblastoma (AVAglio) and RTOG-0825 studies evaluated first-line radiotherapy, temozolomide, and either bevacizumab or placebo in newly diagnosed gliomas; AVAglio reported median progression-free survival (PFS) of 10.6 versus 6.2 months, respectively,[14] and RTOG-0825 reported median PFS of 10.7 versus 7.3 months, respectively.[15] However, neither study demonstrated an OS benefit for the bevacizumab-containing regimen. A retrospective subgroup analysis of AVAglio reported that mesenchymal and proneural subtypes derived PFS benefit with the bevacizumab combination; however, only the proneural subtype reported a survival benefit.[16]

The mesenchymal-epithelial transition factor (MET) pathway can promote proliferation, survival, and metastasis in tumors and can be dysregulated by MET receptor mutations or amplification, and overexpression of its ligand, hepatocyte growth factor (HGF).[17] Murine models suggest that the MET pathway may lead to resistance in glioblastomas receiving antiangiogenic therapy.[18] Glioblastomas can express MET as assessed by immunohistochemistry (IHC)[19,20]; approximately 88% have aberrant downstream MET signaling, and 4% have *MET* amplification.[21] Expression of HGF is also seen in glioblastomas.[21,22] Onartuzumab, a humanized, monovalent monoclonal anti-MET antibody, demonstrated inhibition of glioblastoma growth in preclinical testing.[23] Therefore, the MET pathway is a rational target to investigate in glioblastoma. The phase II GO27819 study (NCT01632228) investigated the safety and efficacy of onartuzumab plus bevacizumab versus placebo plus bevacizumab in recurrent glioblastoma.

PATIENTS AND METHODS

At first recurrence after chemoradiation, bevacizumab-naïve patients with glioblastoma were randomly assigned 1:1 by interactive voice/Web response system to receive intravenous onartuzumab (15 mg/kg) plus bevacizumab (15 mg/kg; Ona + Bev) or placebo plus bevacizumab (Pla + Bev) in 3-weekly cycles (day 1) until disease progression. Enrollment in a Pla + Ona arm was put on hold during an initial safety assessment and was subsequently closed as a result of concerns regarding unequal randomization. Patients were stratified by age ($<$ 50 years *v* $\geq$ 50 years) and Karnofsky performance status (70% to 80% *v* 90% to 100%).

To meet inclusion criteria, patients had to be at least 18 years old with Karnofsky performance status $\geq$ 70%; with histologically confirmed glioblastoma at first recurrence (by Response Assessment in Neuro-Oncology criteria[24]) after concurrent or adjuvant chemoradiotherapy (no more than one previous temozolomide-based regimen; no previous therapy targeting angiogenic or MET pathways). Prior therapy with Gamma Knife or focal high-dose radiotherapy was allowed if histologic recurrence was documented.

Patients were excluded if they were unable to undergo brain magnetic resonance imaging scans with intravenous gadolinium. Patients with absolute neutrophil count $<$ 1.5 $\times$ 10^9/L, platelet count $<$ 100 $\times$ 10^9/L, hemoglobin $<$ 9.0 g/dL, total bilirubin $\geq$ 1.5 $\times$ upper limit of normal, serum creatinine $>$ 1.5 $\times$ upper limit of normal, calculated creatinine clearance $<$ 60 mL/min, or urine dip-stick test for proteinuria $\geq$ 2+ were also excluded. Patients who had prior treatment with prolifeprospan 20 carmustine wafer or a prior intracerebral agent were excluded. Patients with inadequately controlled hypertension or diabetes, history of hypertensive crisis, hypertensive encephalopathy, or significant vascular disease were not included. Patients with evidence of bleeding diathesis or coagulopathy, history of abdominal fistula, GI perforation or intracranial abscess within 6 months before random assignment, or history of another malignancy in the previous 3 years (with a disease-free interval of $<$ 3 years) were also excluded, as were those with serious nonhealing wounds, active ulcers, or untreated bone fractures.

Paraffin-embedded tumor samples were required to determine MET IHC expression status.[25] Tissue from recurrent surgery was preferred, but tissue from the initial surgery was sufficient for study entry. MET-positive status was initially defined as $\geq$ 50% of tumor cells with membrane and/or cytoplasmic staining at moderate-to-high intensity. An exploratory MET-positive status cutoff of $\geq$ 10% was also evaluated.

HGF RNA expression was assessed by cobas polymerase chain reaction (PCR; Roche Molecular Systems, Pleasanton, CA), with high expression classed as the upper 25% subgroup and low expression as the lower 75% subgroup on the basis of analysis of the Subpopulation Treatment Effect Pattern Plots (Appendix Fig A1, online only). *HGF* expression was also evaluated by nonisotopic in situ hybridization (ISH) using branched DNA technology (Advanced Cell Diagnostics, Hayward, CA; Appendix Fig A2, online only). Promoter methylation of DNA repair gene O^6-methylguanine–DNA methyltransferase (*MGMT*), a potential positive prognostic marker for glioblastomas,[26] was also evaluated by quantitative methylation-specific PCR.[27] Retrospective analyses of AVAglio indicated that glioblastoma subtypes were differentially associated with bevacizumab efficacy[16]; therefore, in this analysis, correlation between glioblastoma subtype determined by NanoString gene expression and efficacy was assessed (Appendix, online only).

The coprimary end points were PFS by Response Assessment in Neuro-Oncology criteria in the intent-to-treat (ITT) population and the MET-positive subpopulation. Secondary end points (for ITT and MET biomarker–positive subgroups) included median OS, 9-month OS rate, 6-month PFS rate, overall response rate (ORR), duration of response, and safety. PFS and OS were assessed using the Kaplan-Meier method. *P* values of Ona + Bev versus Pla + Bev were obtained from either the log-rank test or the Cox model, and hazard ratios (HRs) were estimated from the Cox model. The biomarker effect was further evaluated using the multivariable Cox model, including the treatment, biomarker, their interaction, and baseline characteristics. Statistical significance was set at .05 (two-sided significance). Safety was assessed per National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0. The study was conducted in 42 centers across eight countries, in accordance with the Declaration of Helsinki and Good Clinical Practice principles; patients provided written informed consent.

RESULTS

Patients

Between June 2012 and February 2013, 64 patients were randomly assigned to Ona + Bev and 65 to Pla + Bev. Patient disposition is shown in Fig 1. The clinical data cutoff was November 7, 2013. The median follow-up was 9.8 and 9.9 months, respectively, for Ona + Bev and Pla + Bev. At the clinical cutoff, 59 patients receiving Ona + Bev (92%) and 55 patients receiving Pla + Bev (85%) had stopped study treatment (Fig 1).

Baseline characteristics were balanced between the arms (Table 1). Most patients (91.5%) had tumors with low MET expression (MET IHC score 0, 1+; IHC 50% staining cutoff). For the 10% staining cutoff, 79.8% of tumors were MET negative. Because only five patients across the arms had MET-positive tumors by the 50% staining cutoff, this sample was deemed too small to produce meaningful results; therefore, the planned analyses in this subgroup were cancelled.

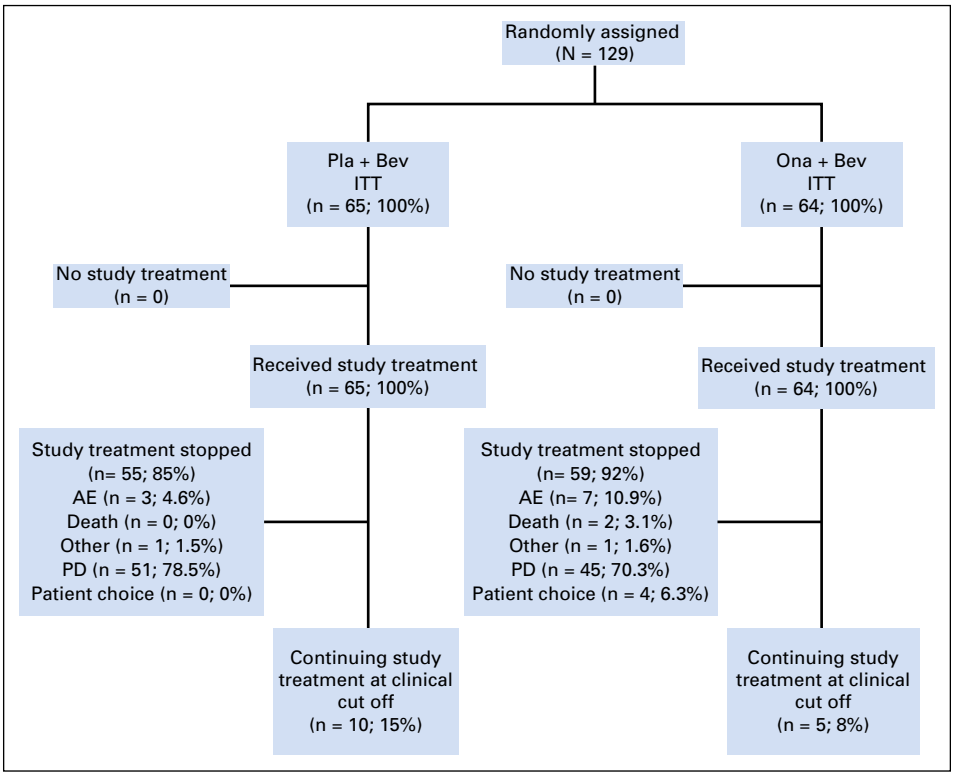

Fig 1. CONSORT diagram. Patient disposition. AE, adverse event; ITT, intent to treat; Ona + Bev, onartuzumab plus bevacizumab; PD, progressive disease; Pla + Bev, placebo plus bevacizumab.

Efficacy

The median PFS (ITT) was 3.9 months for Ona + Bev and 2.9 months for Pla + Bev (HR, 1.06; 95% CI, 0.72 to 1.56; $P = .7444$; Fig 2A). Subgroup analyses of PFS were consistent with ITT data (Fig 2B). The median OS was 8.8 months for Ona + Bev and 12.6 months for Pla + Bev (HR, 1.45; 95% CI, 0.88 to 2.37; $P = .1389$; Fig 2C). Predefined subgroup analyses of OS were generally consistent with ITT results (Fig 2D). The 6-month PFS rate was 33.9% for Ona + Bev and 29.0% for Pla + Bev ($P = .5555$). The 9-month OS rates were 49.7% and 57.2%, respectively, for Ona + Bev and Pla + Bev ($P = .4115$). The ORR was 22.2% in the Ona + Bev arm (12 of 54 patients; one complete response) and 23.7% in the Pla + Bev arm (14 of 59 patients; three complete responses). The median duration of response was 6.4 months for Ona + Bev and 9.7 months for Pla + Bev.

Exploratory Biomarker Analysis

Glioblastoma subtype. Recent retrospective analysis from AVAglio indicated that glioblastoma subtypes were differentially associated with outcomes to bevacizumab[16]; therefore, glioblastoma subtype was evaluated in GO27819. There were proportionally more mesenchymal-subtype tumors (47% *v* 41%, respectively) and fewer proneural tumors (23.5% *v* 30.3%, respectively) in the GO27819 study than in AVAglio. The results showed that there was no difference in PFS between the mesenchymal subtype compared with other subtypes in GO27819 (Appendix Fig A3, online only).

Because MET/HGF signaling is essential in maintaining the mesenchymal phenotype, the association between *HGF* expression and glioblastoma subtype was analyzed in AVAglio and GO27819 using NanoString technology.[16] As expected, *HGF* expression was associated with the mesenchymal phenotype in both studies, suggesting that this may be a common occurrence in glioblastoma biology (Appendix Tables A1 to A3, online only).

HGF Analysis

Analysis of *HGF* expression in the AVAglio study showed no significant differences in PFS (HR, 1.25; $P = .24$) or OS (HR, 1.11; $P = .57$) for high expression of *HGF* versus low expression of *HGF* in the control arm; however, low expression of *HGF* seemed to be predictive of improved efficacy for bevacizumab (PFS: HR, 1.71; $P = .0056$; OS: HR, 1.56; $P = .027$; Appendix Fig A4, online only). Therefore, it was decided to correlate *HGF* expression with efficacy in GO27819.

In GO27819, 119 patients (Ona + Bev, n = 58; Pla + Bev, n = 61) had PCR results for *HGF* available. In the Pla + Bev arm, patients with high *HGF* expression (upper 25%, n = 16) had shorter efficacy outcomes than those with lower PCR levels of *HGF* (lower 75%, n = 45) for PFS (median PFS: 2.8 *v* 4.1 months, respectively; HR, 1.67; 95% CI, 0.90 to 3.13; $P = .1167$) and OS (median OS: 7.3 months for the upper 25% group and not reached [NR] for the lower 75% group; HR, 1.65; 95% CI, 0.76 to 3.59; $P = .2012$); however, the differences were not statistically significant.

In patients with PCR expression of *HGF* in the upper 25% subgroup (Ona + Bev, n = 14; Pla + Bev, n = 16), significantly longer median PFS was seen with Ona + Bev (6.1 months) versus Pla + Bev (2.8 months; HR, 0.37; 95% CI, 0.16 to 0.86; $P = .0201$; Fig 3A). The median OS was longer with Ona + Bev in this subgroup (NR for Ona + Bev *v* 7.3 months for Pla + Bev; HR, 0.29; 95% CI, 0.08 to 1.06; $P = .0604$; Fig 3B). Patients in the upper 25% subgroup of PCR

Table 1. Baseline Patient Characteristics

Characteristic	Ona + Bev (n = 64)	Pla + Bev (n = 65)
Median age, years	57.0	55.0
Age < 50 years	18 (28.1)	17 (26.2)
Age ≥ 50 years	46 (71.9)	48 (73.8)
Sex		
Male	44 (68.8)	39 (60.0)
Female	20 (31.3)	26 (40.0)
Karnofsky performance status		
70%-80%	36 (56.3)	37 (56.9)
90%-100%	28 (43.8)	28 (43.1)
Race		
Asian	3 (4.7)	0 (0.0)
White	59 (92.2)	64 (98.5)
Other	2 (3.1)	0 (0.0)
Multiple	0 (0.0)	1 (1.5)
Corticosteroid use at baseline	10 (15.6)	15 (23.1)
Prior surgery		
Biopsy	5 (7.8)	2 (3.1)
Complete resection	32 (50.0)	38 (58.5)
Partial resection	26 (40.6)	23 (35.4)
Measurable disease at baseline*	54 (84.4)	59 (90.8)
Number of target lesions at baseline		
≤ 1	39 (60.9)	47 (72.3)
> 1	15 (23.4)	12 (18.5)
Glioblastoma subtype	n = 58	n = 61
Mesenchymal	27 (46.6)	29 (47.5)
Proneural	14 (24.1)	14 (23.0)
Proliferative	10 (17.2)	10 (16.4)
Unclassified	7 (12.1)	8 (13.1)
MET IHC score (50% staining cutoff)		
0	52 (81.3)	53 (81.5)
1+	4 (6.3)	9 (13.8)
2+	3 (4.7)	1(1.5)
3+	0 (0.0)	1 (1.5)
MET IHC (10% staining cutoff)		
0	35 (54.7)	35 (53.8)
1+	17 (26.6)	16 (24.6)
2+	7 (10.9)	11 (16.9)
3+	0 (0.0)	2 (3.1)
HGF PCR status	n = 58	n = 61
Upper 25%	14 (24.1)	16 (26.2)
Lower 75%	44 (75.9)	45 (73.8)
HGF ISH score		
0	16 (25.0)	17 (26.2)
1+	21 (32.8)	25 (38.5)
2+	16 (25.0)	18 (27.7)
3+	4 (6.3)	3 (4.6)
MGMT methylation status	n = 56	n = 54
Methylated	21 (37.5)	26 (48.1)
Unmethylated	32 (57.1)	25 (46.3)
Possible, weak methylation	3 (5.4)	3 (5.6)
IDH1 mutation status	n = 58	n = 57
Mutation-positive	4 (6.9)	5 (8.8)
Wild-type	54 (93.1)	57 (91.2)

NOTE. Data presented as No. (%) unless otherwise noted.
Abbreviations: Bev, bevacizumab; *HGF*, hepatocyte growth factor; *IDH1*, isocitrate dehydrogenase 1; IHC, immunohistochemistry; ISH, in situ hybridization; MET, mesenchymal-epithelial transition factor; *MGMT*, O6-methylguanine–DNA methyltransferase; Ona, onartuzumab; PCR, polymerase chain reaction; Pla, placebo.
*Confirmation of measurable disease was not a protocol-defined inclusion criterion.

expression of *HGF* also had significantly higher ORR with Ona + Bev compared with Pla + Bev (35.7% *v* 0%; *P* = .014).

Among patients with PCR expression of *HGF* in the lower 75% subgroup, those treated with Ona + Bev (n = 44) had shorter median PFS (2.8 *v* 4.1 months, respectively; HR, 1.39; 95% CI, 0.87 to 2.20; *P* = .1589) and significantly shorter OS (8.6 months *v* NR, respectively; HR, 1.86; 95% CI, 1.03 to 3.36; *P* = .0381) compared with those treated with Pla + Bev (n = 45; Fig 3C and D).

HGF expression was also assessed by ISH in GO27819 and correlated with PFS and OS results (Appendix Fig A5, online only). The overlap of high versus low *HGF* expression as assessed by PCR versus ISH is shown in Appendix Table A4 (online only).

Although the mesenchymal subtype did not predict outcomes, *HGF* levels seemed to be predictive of Ona + Bev efficacy. To evaluate this, we compared outcomes for the mesenchymal subtype or other subtypes stratified by *HGF* expression (Appendix Fig A6, online only). There was no difference in outcomes between the arms for the mesenchymal subtype with low *HGF* expression, whereas patients with high *HGF* expression seemed to derive benefit from the addition of onartuzumab to bevacizumab. There were no differences in outcomes for patients in the nonmesenchymal subset with low *HGF* expression. The nonmesenchymal subset of patients with high *HGF* expression was too small (n = 9) to robustly evaluate efficacy.

MGMT *Methylation Analysis*

Methylation of *MGMT* is a well-known prognostic marker in glioblastoma. Thus 110 patients (Ona + Bev, n = 56; Pla + Bev, n = 54) were analyzed for *MGMT* methylation status. Forty-seven patients had methylated *MGMT*, 57 had unmethylated *MGMT*, and six patients had unconfirmed *MGMT* status. Assessing the Pla + Bev arm only, patients had a worse outcome when treated with Pla + Bev if they had unmethylated *MGMT* and a better prognosis if they had methylated *MGMT* (PFS: HR, 3.19; 95% CI, 1.58 to 6.44; OS: HR, 3.39; 95% CI, 1.32 to 8.75; Appendix Fig A7, online only).

Median PFS and OS were longer with Ona + Bev (n = 32) compared with Pla + Bev (n = 25) in patients with unmethylated *MGMT* (Fig 4); the median PFS was 4.2 *v* 2.8 months, respectively (HR, 0.46; 95% CI, 0.25 to 0.84; *P* = .0108) and the median OS was 10.9 *v* 7.5 months, respectively (HR, 0.53; 95% CI, 0.26 to 1.10; *P* = .0836). A numerically higher ORR was seen with Ona + Bev compared with Pla + Bev in the unmethylated *MGMT* subgroup (15.6% *v* 8.0%; *P* = .450). Among patients with methylated *MGMT*, those treated with Ona + Bev (n = 21) had shorter median PFS compared with patients treated with Pla + Bev (n = 26; 2.8 *v* 6.4 months, respectively; HR, 1.52; 95% CI, 0.75 to 3.08; *P* = .2440) and significantly shorter median OS (7.7 months *v* NR, respectively; HR, 3.18; 95% CI, 1.19 to 8.51; *P* = .0150).

Results of multivariable analyses of the *HGF* and *MGMT* biomarkers are shown in Appendix Table A5 (online only). With adjustment by the baseline characteristics, MGMT status and PCR analysis of HGF still result in statistically significant effects for PFS and OS by *MGMT* methylation, and PFS by high or low HGF expression. There was also a potentially predictive treatment effect of Ona + Bev for patients with unmethylated *MGMT* or with high HGF expression by PCR. The multivariable results were generally consistent with the subgroup analyses.

Correlation between *MGMT* methylation and PFS benefit with bevacizumab in AVAglio was also investigated. No predictive influence of *MGMT* methylation status on PFS benefit

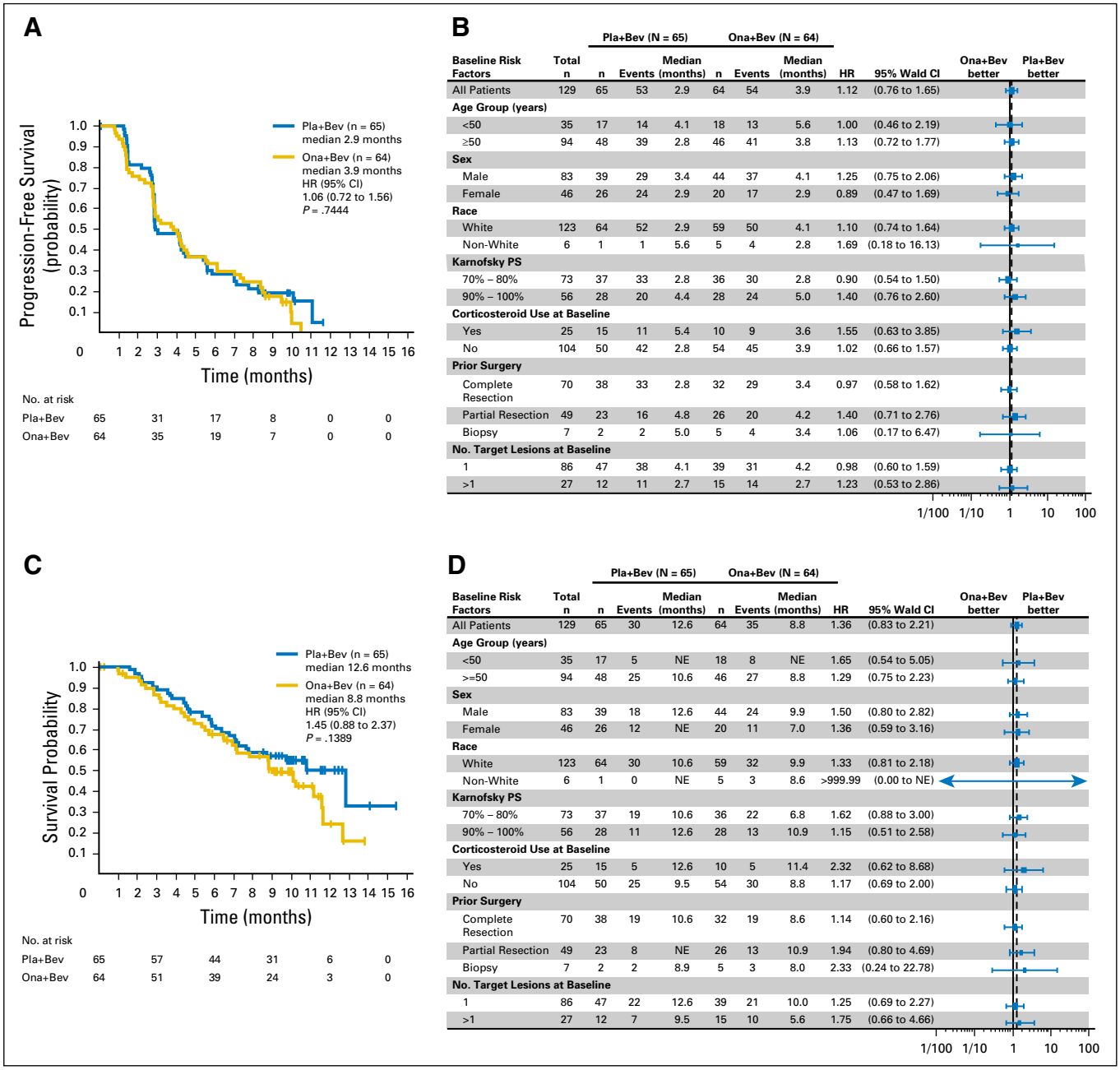

Fig 2. (A) Progression-free survival in the intent-to-treat population and (B) forest plot of progression-free survival subgroup analysis; (C) overall survival in the intent-to-treat population and (D) forest plot of overall survival subgroup analysis. HR, hazard ratio; NE, non-evaluable; Ona + Bev, onartuzumab plus bevacizumab; Pla + Bev, placebo plus bevacizumab; PS, performance status.

with bevacizumab was observed (HR, 0.76 for methylated; HR, 0.56 for unmethylated).[14]

Safety

The median number of cycles for bevacizumab was five for Ona + Bev and six for Pla + Bev; the median number of cycles for onartuzumab was four and six for placebo. The most common classes of adverse events (AEs; any grade) were general disorders (70.8% Ona + Bev *v* 57.8% Pla + Bev), including peripheral edema (44.6% *v* 14.1%), asthenia (24.6% *v* 21.9%), and fatigue (15.4% *v* 20.3%); and nervous system disorders (49.2% Ona + Bev *v* 75.0% Pla + Bev), including headaches (15.4% *v* 23.4%). Grade ≥ 3 AEs were reported in 38.5% (n = 25) of patients receiving Ona + Bev and 35.9% (n = 23) of those receiving Pla + Bev, including nervous system disorders (7.7% *v* 18.8%), vascular disorders (4.6% *v* 6.3%), and respiratory disorders (4.6% *v* 1.6%). Serious AEs were reported in 30.8% and 29.7% of patients, respectively, for Ona + Bev and Pla + Bev (Table 2). Grade 5 AEs were reported for two patients receiving Ona + Bev (two cases of intestinal perforation) and one patient receiving Pla + Bev (intracranial hemorrhage). AEs (all grades) with a difference in incidence ≥ 10% between the arms

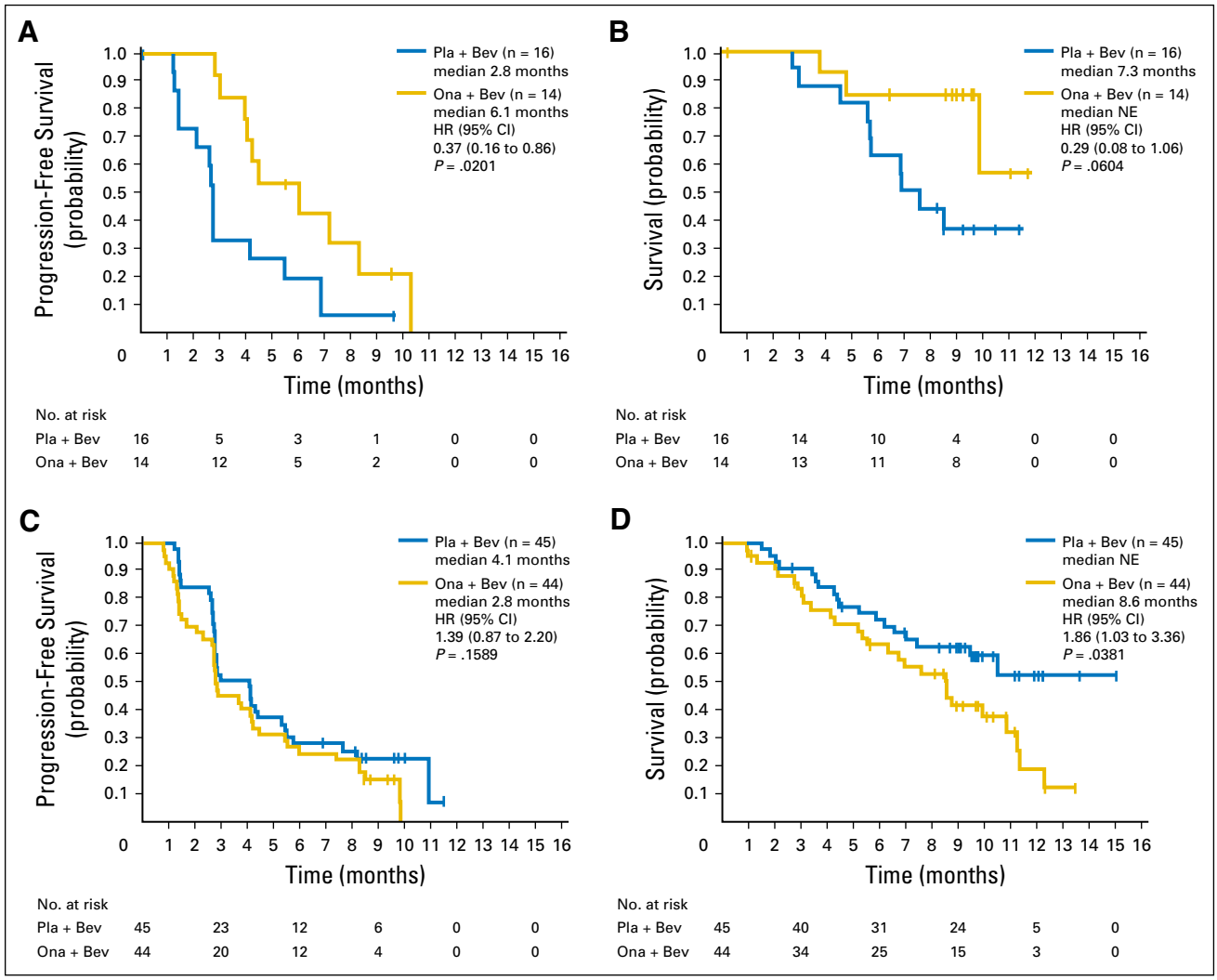

Fig 3. (A) Progression-free survival and (B) overall survival in the polymerase chain reaction levels of hepatocyte growth factor in the upper 25% subgroup; (C) progression-free survival and(D) overall survival in the polymerase chain reaction levels of hepatocyte growth factor in the lower 75% subgroup. HR, hazard ratio; NE, non-evaluable; Ona + Bev, onartuzumab plus bevacizumab; Pla + Bev, placebo plus bevacizumab.

were peripheral edema (44.6% Ona + Bev *v* 14.1% Pla + Bev), hypertension (12.3% Ona + Bev *v* 32.8% Pla + Bev), and hypoalbuminemia (12.3% Ona + Bev *v* 1.6% Pla + Bev).

DISCUSSION

Because recurrent glioblastoma has no standard treatment,[28] novel treatment combinations are continually being investigated. GO27819 evaluated the antiangiogenic agent bevacizumab plus the anti-MET antibody onartuzumab in recurrent glioblastoma.

The GO27819 study reported a similar median PFS for Ona + Bev relative to the Pla + Bev control. The median PFS with Ona + Bev (3.9 months) was similar to that observed with bevacizumab plus lomustine (4.0 months) in the BELOB study.[12] The phase II BRAIN study of bevacizumab plus irinotecan reported slightly longer median PFS (5.6 months for bevacizumab plus irinotecan *v* 4.2 months for bevacizumab alone)[7] than that reported in GO27819. A median PFS of 3.6 months and a 6-month PFS rate of 18.8% was reported for a combination of bevacizumab with temozolomide.[13] Therefore, the PFS results in GO27819 are in the range of those previously reported for bevacizumab-based combination regimens for treatment of recurrent glioblastoma. However, it is important to note that cross-trial comparisons should be evaluated with caution, because there may be differences in patient populations and neuroimaging assessment methods.

A planned efficacy analysis by MET IHC status could not be performed because of the low prevalence of MET-positive tumors, a limitation of this study. This could have been avoided if the study had been designed to enrich for MET-positive tumors, enrolling patients until an adequate number of MET-positive samples were available for analysis. The use of tissue from diagnosis, rather than from the time of recurrence, to assess MET status may also be considered a limitation.

In an exploratory biomarker analysis, *HGF* levels, *MGMT* promoter methylation status, and glioblastoma subtype were correlated with efficacy outcomes. High *HGF* levels by PCR or ISH seemed to be prognostic for worse OS and PFS, but potentially

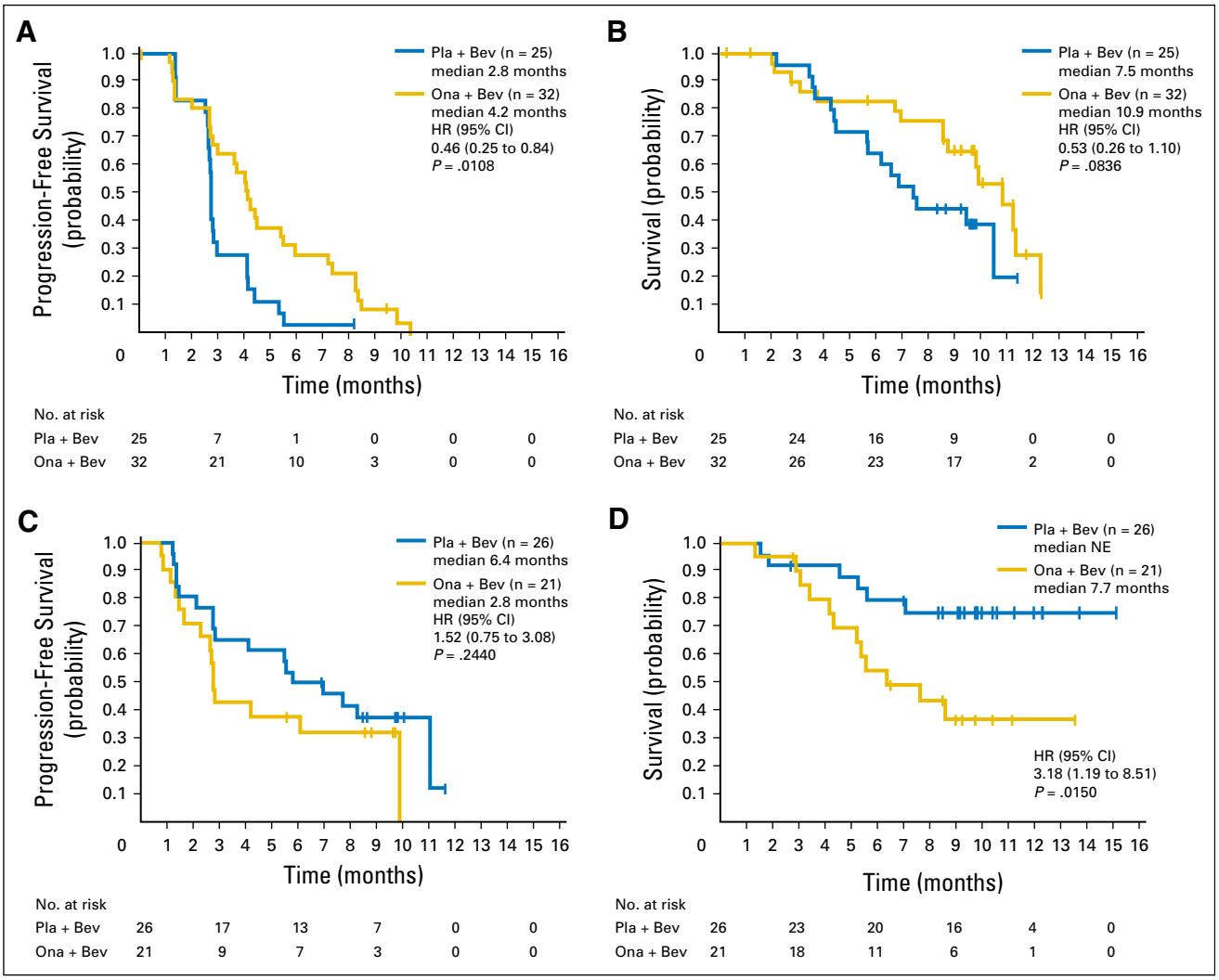

Fig 4. (A) Progression-free survival and (B) overall survival in the O^6-methylguanine–DNA methyltransferase unmethylated subgroup; (C) progression-free survival and (D) overall survival in the O^6-methylguanine–DNA methyltransferase methylated subgroup. HR, hazard ratio; NE, non-evaluable; Ona + Bev, onartuzumab plus bevacizumab; Pla + Bev, placebo plus bevacizumab.

predictive of superior efficacy with Ona + Bev compared with Pla + Bev. High *HGF* expression may indicate an aberrant MET pathway; in gastric cancer models, expression of *HGF* correlates with MET pathway activity and can predict efficacy of anti-MET agents.[29] In hepatocellular carcinoma, circulating *HGF* levels correlate with decreased survival in untreated patients.[30]

Analysis of *HGF* in the phase III AVAglio study of radiotherapy, temozolomide plus bevacizumab or placebo in newly diagnosed gliomas showed that the mesenchymal subtype was associated with high *HGF* expression and reported that patients with the mesenchymal subtype had longer OS than those with other subtypes.[16] In this onartuzumab study, *HGF* expression was associated with the mesenchymal subtype; however, not all mesenchymal tumors expressed high *HGF* levels and the mesenchymal phenotype was not associated with different outcomes compared with other phenotypes, most likely as a result of the bevacizumab backbone in both treatment arms.

Tumor-specific promoter methylation and silencing of *MGMT* enhances the efficacy of alkylating agents in tumor cells because the cells are unable to efficiently repair damage induced by these agents.[26] The exploratory results in GO27819 suggest that the lack of *MGMT* methylation may be predictive for Ona + Bev outcomes (PFS and OS) in glioblastoma, although the OS results were not statistically significant. However, in the AVAglio study, no predictive influence of *MGMT* methylation status was observed on bevacizumab efficacy.[14] *MGMT* silencing via methylation allows alkylating agents to cause cell death by crosslinking, thereby improving efficacy of these agents.[26] In the absence of a direct biologic link between *MGMT* methylation and MET, other than a potentially additive effect on the apoptosis pathway, it is notable that the treatment effect with unmethylated *MGMT* was similar to the high *HGF* subgroup. Clearly, some potent prognostic factors influence the pathogenesis of glioblastoma, which need to be considered in future trial designs. Therefore, it is essential to appropriately size studies to enable evaluation of exploratory hypotheses.

Although the biomarker data presented above are compelling and should inform future trials targeting the MET pathway, their

Table 2. Safety Analysis

AEs	Ona + Bev (n = 65)*	Pla + Bev (n = 64)*
Grade ≥ 3 AEs	25 (38.5)	23 (35.9)
Serious AEs	20 (30.8)	19 (29.7)
Nervous system disorders	5 (7.7)	11 (17.2)
Vascular disorders	4 (6.2)	5 (7.8)
Infections and infestations	4 (6.2)	4 (6.3)
GI disorders	4 (6.2)	0 (0.0)
Grade 5 AEs	2 (3.1)	1 (1.6)
AEs leading to withdrawal of any study drug	7 (10.8)	4 (6.3)
AEs leading to dose interruption of any study drug	21 (32.3)	15 (23.4)

NOTE. All values are expressed as no. (%).
Abbreviations: AE, adverse event; Bev, bevacizumab; Ona, onartuzumab; Pla, placebo.
*On the basis of treatment received if different than treatment randomly assigned to receive.

exploratory nature and relatively small sample size make it difficult to infer firm conclusions on the clinical utility of *HGF* expression or *MGMT* methylation as predictive markers in glioblastoma. Future prospective studies are necessary to validate these results.

In conclucion, in the GO27819 trial, there was no evidence of improved clinical benefit with the addition of onartuzumab to bevacizumab compared with bevacizumab plus placebo in ITT patients with recurrent glioblastoma. However, exploratory biomarker analyses suggested that patients with high *HGF* expression or unmethylated *MGMT* may benefit from onartuzumab plus bevacizumab.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at ascopubs.org/journal/jco.

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Randomized, Double-Blind, Placebo-Controlled, Multicenter Phase II Study of Onartuzumab Plus Bevacizumab Versus Placebo Plus Bevacizumab in Patients With Recurrent Glioblastoma: Efficacy, Safety, and Hepatocyte Growth Factor and O^6-Methylguanine–DNA Methyltransferase Biomarker Analyses

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Appendix

Gene Expression Analysis on the NanoString Platform

Transcript levels in formalin-fixed paraffin-embedded material were assayed on the NanoString gene expression platform (NanoString Technologies, Seattle, WA), as described previously.[16] Data preprocessing was performed using the NanoStringQCPro Bioconductor package (version 1.0.1; http://www.bioconductor.org/packages/release/bioc/html/NanoStringQCPro.html) and the R programming language (version 3.1; http://www.r-project.org). In brief, for each sample, unspecific background was estimated as the mean expression across the negative-control probes and subtracted from the raw counts. Background-corrected counts were $\log^2$ transformed and centered on the median expression of all probes designed to target endogenous transcripts (global median normalization).

Gene Expression Subtype Classification

Subtype classification was performed by assigning each sample to the reference centroid with the highest Pearson correlation across the normalized expression of 31 classifier genes,[16] as described previously (Phillips HS, et al: Cancer Cell 9:157-173, 2006). Samples without positive correlation to any reference centroid remained unclassified.

Differential Hepatocyte Growth Factor (HGF) Expression

Differential expression of HGF between glioblastoma subtypes was assessed by comparing normalized gene expression scores on the $\log^2$ scale. Ninety-five percent CIs for mean HGF expression in each subgroup were estimated on the basis of the Student *t* test distribution. *P* values were obtained by contrasting *HGF* expression in mesenchymal samples with those in each of the other subtypes using a two-sided *t* test.

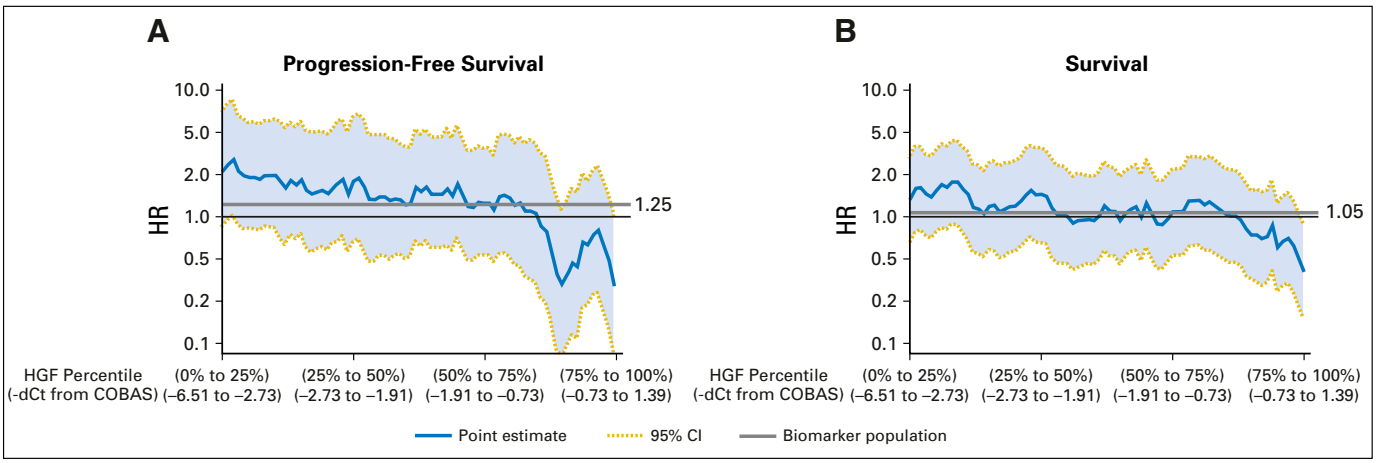

Fig A1. Subpopulation treatment effect pattern plots for hepatocyte growth factor (*HGF*). dCT, delta cycle threshold; HR, hazard ratio.

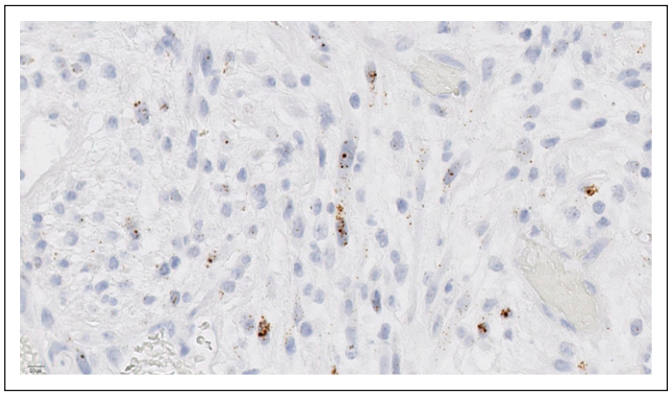

Fig A2. Representative image of hepatocyte growth factor in situ hybridization staining.

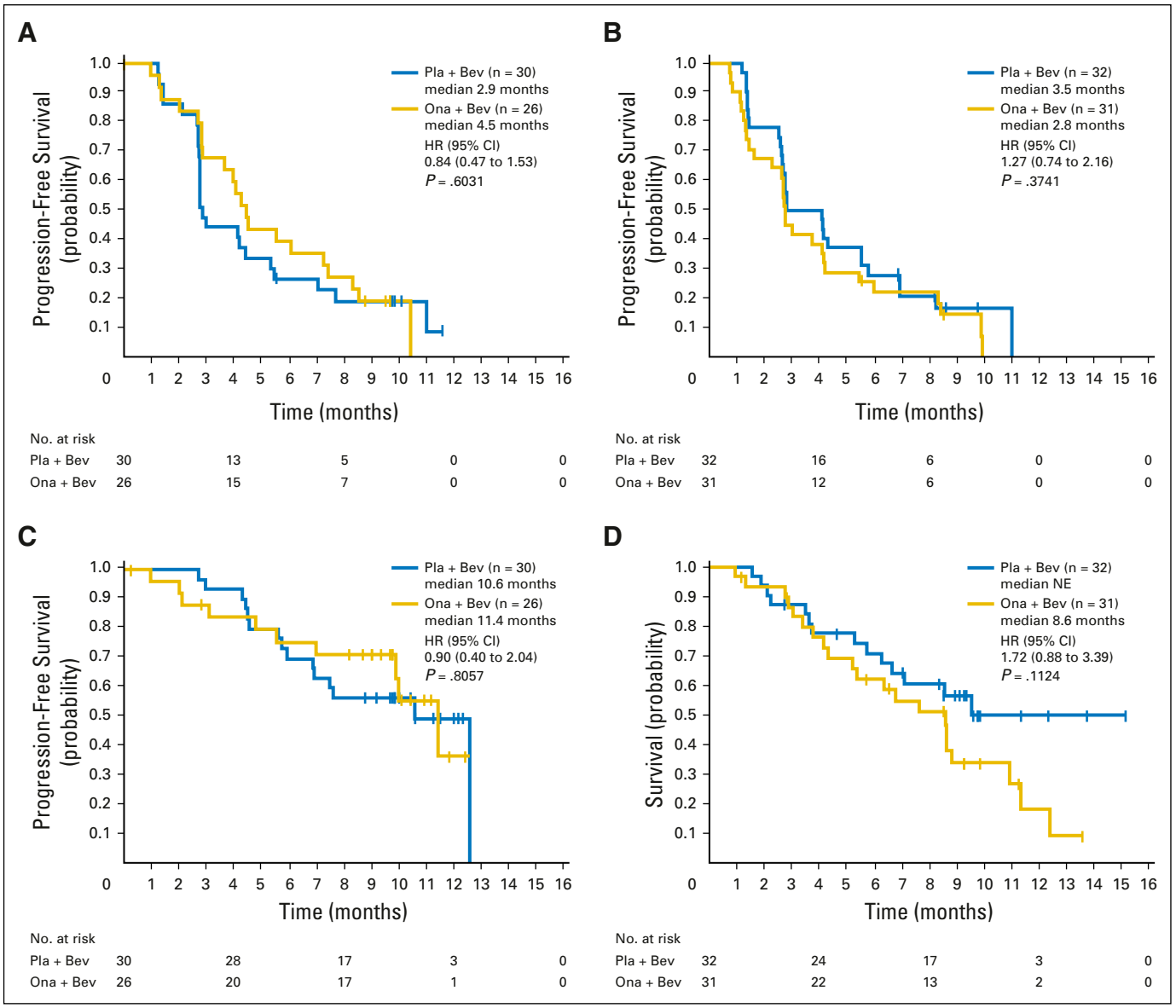

Fig A3. (A) Progression-free survival in mesenchymal subgroup and (B) nonmesenchymal subgroup and (C) overall survival in mesenchymal subgroup and (D) nonmesenchymal subgroup. HR, hazard ratio; NE, non-evaluable; Ona + Bev, onartuzumab plus bevacizumab; Pla + Bev, placebo plus bevacizumab.

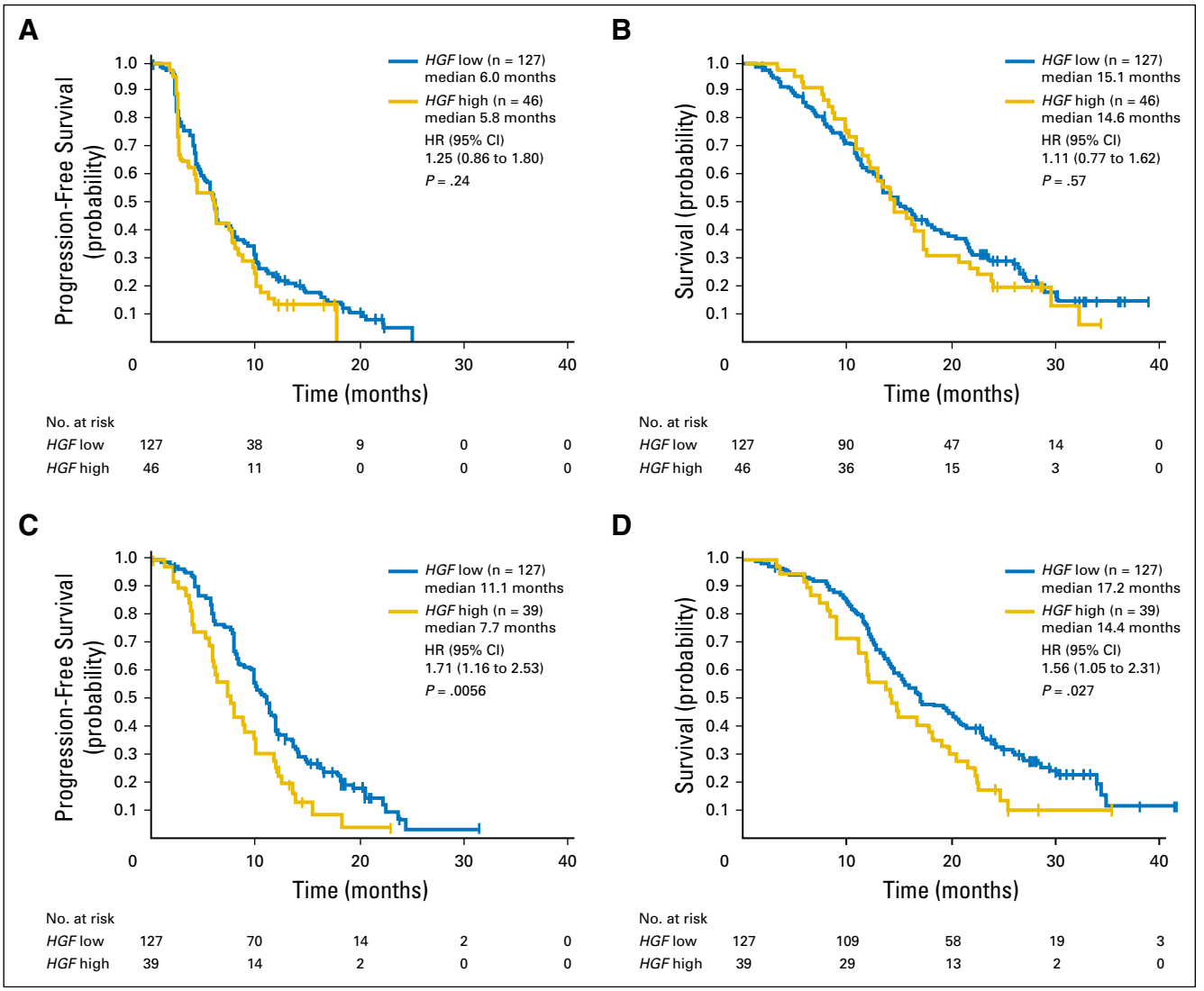

Fig A4. (A) Progression-free survival and (B) overall surviva by NanoString expression of hepatocyte growth factor (*HGF*) with standard of care and (C) progression-free survival and (D) overall survival with standard of care plus bevacizumab in the Avastin in Glioblastoma (AVAglio) study. HR, hazard ratio.

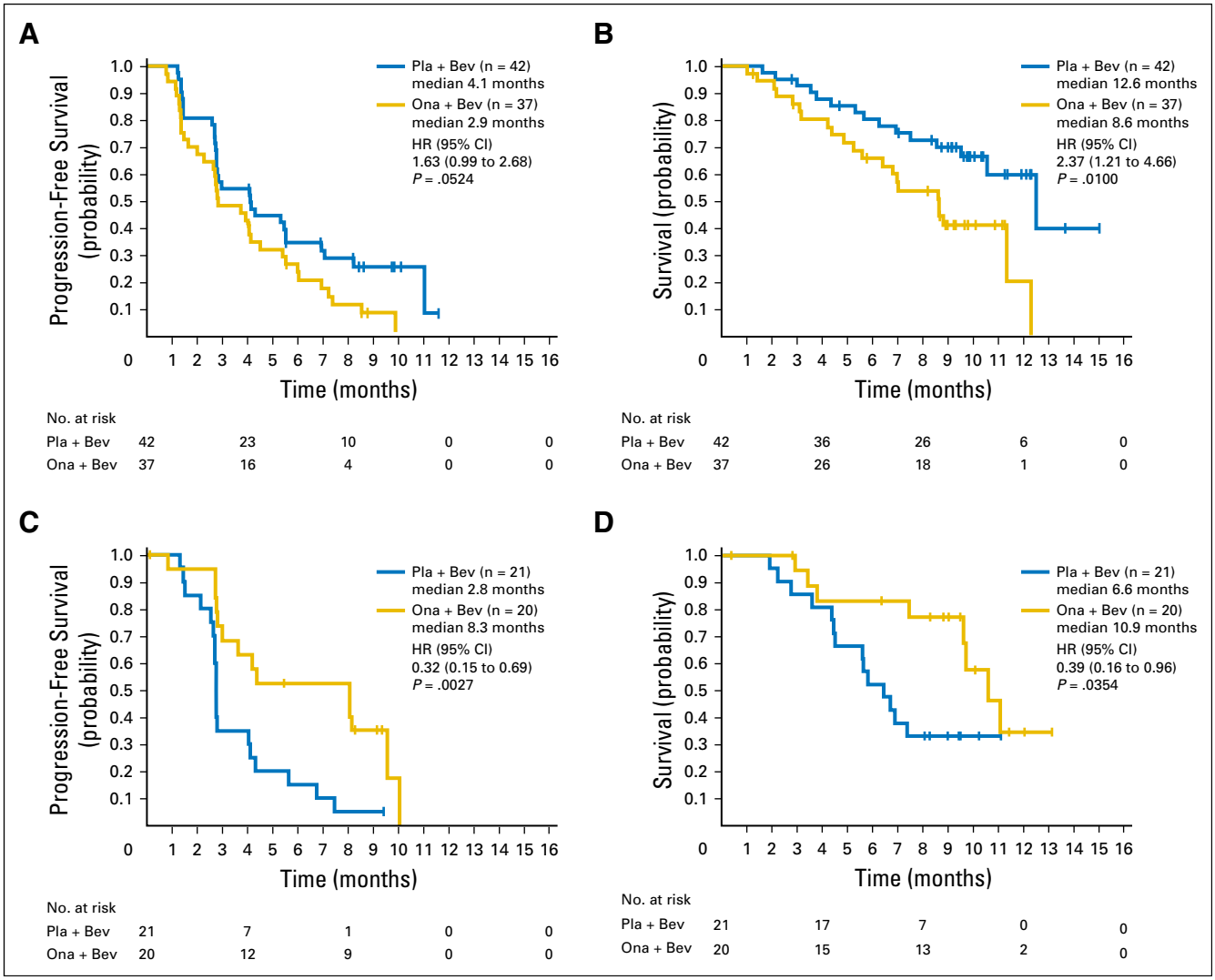

Fig A5. (A) Progression-free survival and (B) overall survival in the low hepatocyte growth factor by in situ hybridization subgroup; (C) progression-free survival and (D) overall survival in the high hepatocyte growth factor by in situ hybridization subgroup. HR, hazard ratio; Ona + Bev, onartuzumab plus bevacizumab; Pla + Bev, placebo plus bevacizumab.

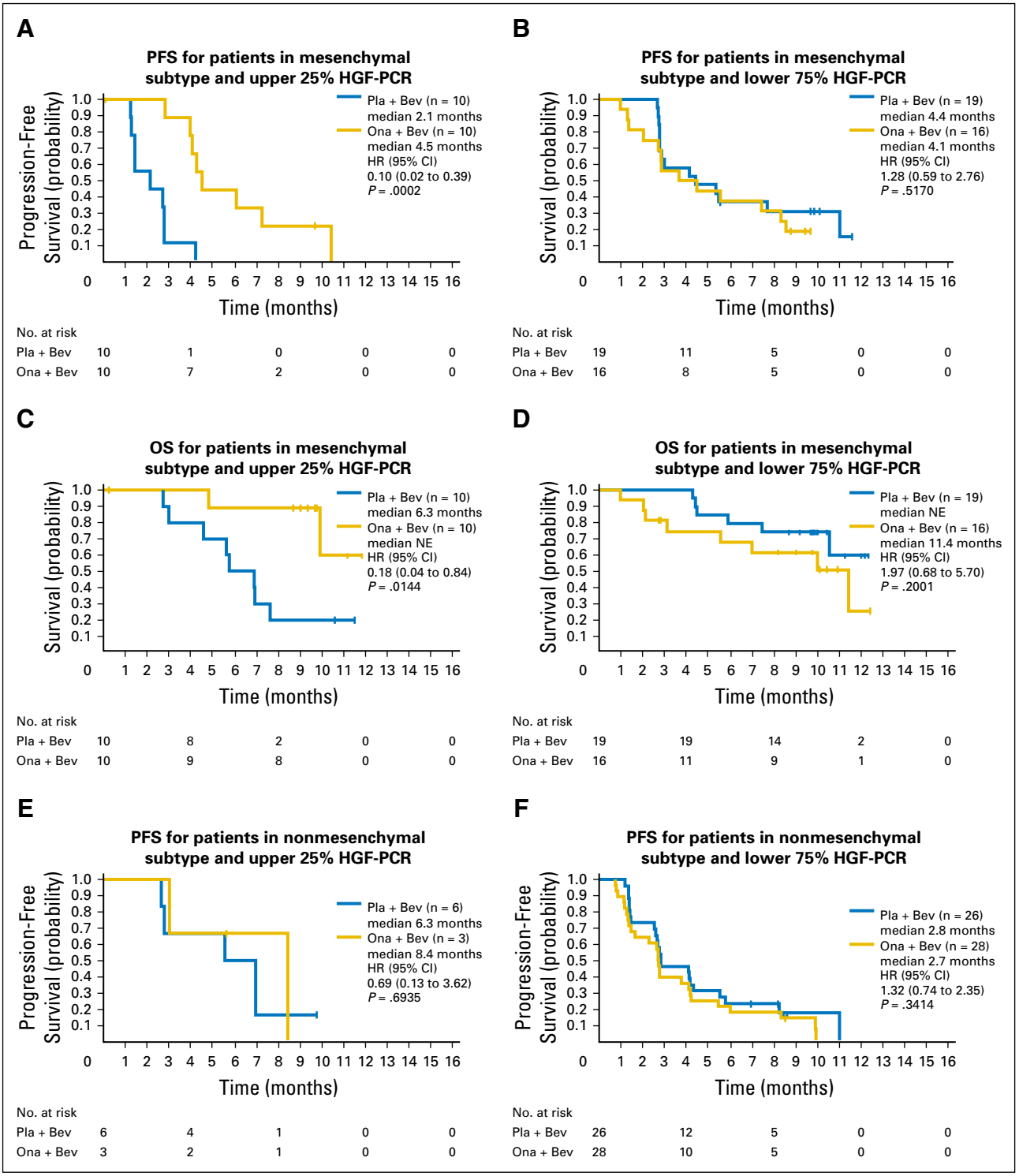

Fig A6. (A to D) Progression-free survival (PFS) and overall survival (OS) in mesenchymal and (E to H) nonmesenchymal subgroups stratified by hepatocyte growth factor (*HGF*) levels. HR, hazard ratio; NE, non-evaluable; Ona + Bev, onartuzumab plus bevacizumab; PCR, polymerase chain reaction; Pla + Bev, placebo plus bevacizumab.

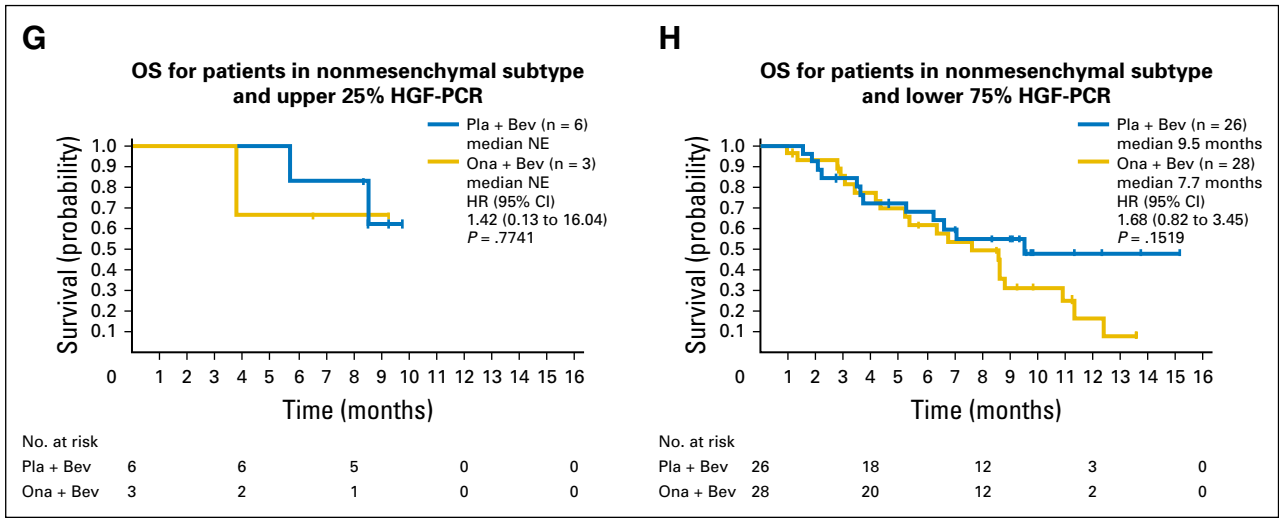

Fig A6. (Continued).

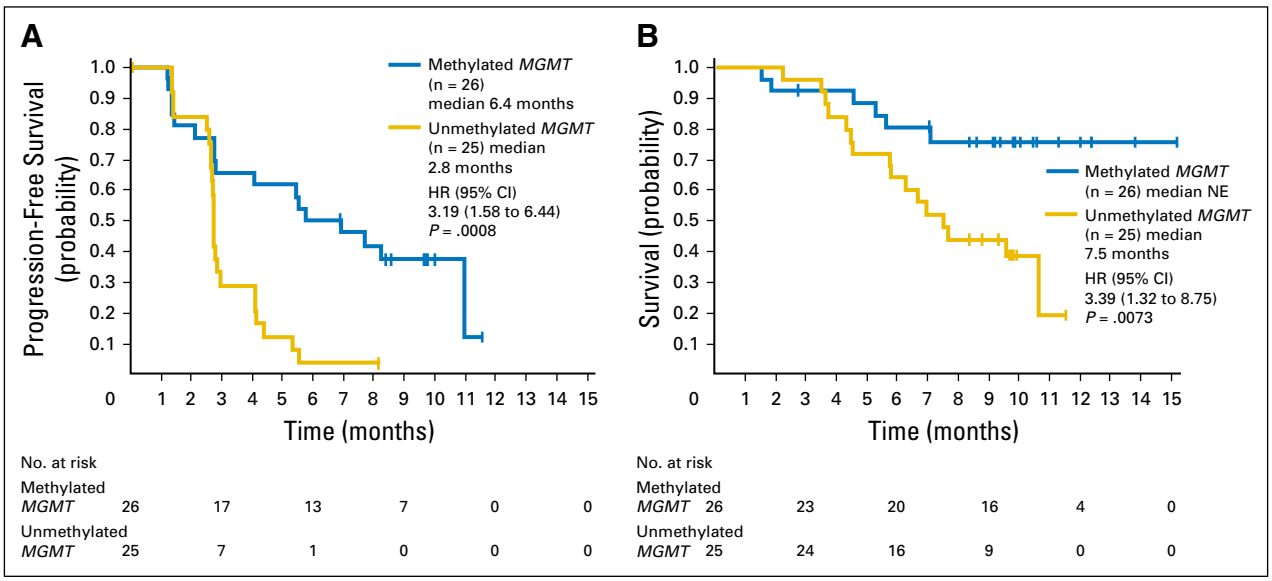

Fig A7. (A) Progression-free survival and (B) overall survival by O^6-methylguanine–DNA methyltransferase (*MGMT*) methylation status in the placebo plus bevacizumab arm. HR, hazard ratio; NE, non-evaluable.

Table A1. Polymerase Chain Reaction Expression of Hepatocyte Growth Factor According to Glioblastoma Subtype in GO27819

Subtype	No.	% Frequency in Sample Population (n = 119)	LSMEAN	95% CI	*P* (ref: mesenchymal)
Mesenchymal	56	47.1	0.620710	0.4997 to 0.7417	
Proliferative	20	16.8	0.198178	−0.0025 to 0.3989	< .001
Proneural	28	23.5	0.336095	0.1665 to 0.5057	.0079
Unclassified	15	12.6	0.377946	0.1462 to 0.6097	.0684

Abbreviation: LSMEAN, least squares mean.

Table A2. NanoString Expression of Hepatocyte Growth Factor According to Glioblastoma Subtype in GO27819

Subtype	No.	% Frequency in Sample Population (n = 119)	LSMEAN	95% CI	*P* (ref: mesenchymal)
Mesenchymal	56	47.1	6.7279	6.3964 to 7.0593	
Proliferative	20	16.8	5.3263	4.8257 to 5.8269	< .001
Proneural	28	23.5	5.9504	5.4167 to 6.4841	.0149
Unclassified	15	12.6	6.4224	5.9212 to 6.9235	.2946

NOTE. Hepatocyte growth factor expression is indicated in normalized $\log^2$ counts.
Abbreviation: LSMEAN, least squares mean.

Table A3. NanoString Expression of Hepatocyte Growth Factor According to Glioblastoma Subtype in Avastin in Glioblastoma (AVAglio) Trial

Subtype	No.	% Frequency in Sample Population (n = 339)	LSMEAN	95% CI	*P* (ref: mesenchymal)
Mesenchymal	139	41.0	6.3524	6.0206 to 6.6842	
Proliferative	58	17.1	5.4113	4.8282 to 5.9944	.0062
Proneural	103	30.3	5.6896	5.2795 to 6.0997	.0136
Unclassified	39	11.5	6.7067	6.1210 to 7.2924	.2933

NOTE. Hepatocyte growth factor expression is indicated in normalized $\log^2$ counts.
Abbreviation: LSMEAN, least squares mean.

Table A4. High and Low Expression of Hepatocyte Growth Factor as Assessed by Polymerase Chain Reaction or In Situ Hybridization

Expression Level	*HGF* by PCR, Upper 25%	*HGF* by PCR, Lower 75%	Total
HGF by ISH, 2+/3+	19 (63.3)	22 (25.3)	41 (35.0)
HGF by ISH, 0/1+	11 (36.7)	65 (74.7)	76 (65.0)
Total	30 (25.6)	87 (74.4)	117

NOTE. All values are expressed as no. (%).
Abbreviations: *HGF*, hepatocyte growth factor; ISH, in situ hybridization; PCR, polymerase chain reaction.

Table A5. Multivariable Analysis of PCR Expression of Hepatocyte Growth Factor and O^6-Methylguanine–DNA Methyltransferase Biomarker Efficacy Outcomes

Ona + Bev *v* Pla + Bev	No.	HR for Treatment	95% CI*	*P* for Biomarker*	Treatment by Biomarker Interaction *P* †
PFS	92			.0182	.173
MGMT		0.685	0.368 to 1.274		
Unmethylated		1.369	0.648 to 2.890		
Methylated					
OS	92			.0409	.0276
MGMT		0.665	0.295 to 1.500		
Unmethylated		3.007	1.095 to 8.257		
Methylated					
PFS	103			.0051	.0011
HGF by PCR		0.224	0.085 to 0.593		
Upper 25%		1.397	0.851 to 2.295		
Lower 75%					
OS	103			.0618	.0077
HGF by PCR		0.201	0.041 to 0.984		
Upper 25%		2.144	1.143 to 4.022		
Lower 75%					

NOTE. Model included Karnofsky performance status (70% to 80% *v* 90% to 100%); number of target lesions at baseline (1 *v* > 1); and glioblastoma subtype (mesenchymal, proneural, proliferative, or unclassified), treatment, biomarker, and interaction term.

Abbreviations: *HGF*, hepatocyte growth factor; HR, hazard ratio; *MGMT*, O^6-methylguanine–DNA methyltransferase; Ona + Bev, onartuzumab plus bevacizumab; OS, overall survival; PCR, polymerase chain reaction; Pla + Bev, placebo plus bevacizumab; PFS, progression-free survival.

*Wald CI/test.

†Likelihood ratio test.